

# Different patterns of molecular, carbon and hydrogen isotope compositions of *n*-alkanes between heterotrophic plant and its hosts

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**Abstract** This study investigates the molecular, carbon, and hydrogen isotope compositions of *n*-alkanes in eight pairs of a holoparasitic plant (*Cuscuta chinensis*) and its hosts. It is unexpected that *C. chinensis* has a higher concentration of *n*-alkanes than its hosts in seven of the eight pairs, and it is preferentially dominated by *n*-C<sub>29</sub> alkane (representing > 75% of the total *n*-alkanes). In addition, the  $\delta^{13}\text{C}$  values of C<sub>29</sub> alkane in *C. chinensis* are less negative (avg. 1.8 ‰) than those in the hosts, while the  $\delta^2\text{H}$  values of C<sub>29</sub> alkane are more negative in *C. chinensis* (the difference averages 48 ‰). We propose that the <sup>2</sup>H-depletion of *n*-alkanes in the stem parasite *C. chinensis* may result from the utilization of stem water with less influence from evapotranspiration or the use of newly synthesized carbohydrates which is <sup>2</sup>H-depleted relative to stored sugars. These results highlight the importance of plant nutrient status on the molecular and isotopic compositions of leaf waxes, which shed light on the (paleo)ecological potential of leaf wax  $\delta^2\text{H}$  values.

**Keywords** heterotrophic plant, *Cuscuta*, *n*-alkane, carbon isotope, hydrogen isotope

## 1 Introduction

Molecular paleoclimate research has made great strides in recent years, establishing a series of paleoclimate indicators and discussing their applicability, helping us to better understand the history of paleoclimatic changes (Eglinton and Eglinton, 2008; Pu et al., 2018; Zang et al., 2018; Liu and An, 2020). *n*-Alkanes are essential components of

plant leaf waxes, acting as an important protection against water loss, and are not easy to degrade in sediments because of their unique molecular structures (Eglinton and Hamilton, 1967; Koch and Ensikat, 2008). The hydrogen isotope composition of leaf wax *n*-alkanes ( $\delta^2\text{H}_{\text{alk}}$ ) has been widely investigated and has been verified as a robust paleohydrological tool for paleoenvironmental reconstruction (Sachse et al., 2012; Sessions, 2016).

Besides the hydrological significance, the ecological potential of  $\delta^2\text{H}_{\text{alk}}$  values has gradually attracted attention (Cormier et al., 2018 and 2019). In the quite limited studies, it is common to observe a <sup>2</sup>H-enrichment of bulk biomass or *n*-alkanes in the obligate heterotrophic plants (Ziegler, 1995; Cormier et al., 2018 and 2019). However, in our recent study, we find a <sup>2</sup>H-depletion of *n*-alkanes in a holoparasitic plant species (*Cuscuta chinensis*). The genera *Cuscuta* (dodder) is distributed worldwide and contains nearly 200 species (Mishra, 2009). All species of *Cuscuta* are annual obligate parasites and uptake nutrients from their hosts via haustorium. *Cuscuta* species are devoid of proper leaves and roots and exhibit very little to no photosynthetic activity. As compared to the heterotrophic species investigated in Cormier et al. (2019), which grows on the roots of hosts or aids by fungi, *Cuscuta* species are stem parasitism. Here we report the molecular, carbon, and hydrogen isotope compositions of *n*-alkanes in specimens of *C. chinensis* and its hosts, intending to elucidate the possible reason responding for the <sup>2</sup>H-depletion in the obligate heterotrophic plant species.

## 2 Materials and methods

During a field trip of late July 2018, eight pairs of *C. chinensis* and hosts were collected along a small mountain

stream and in a nearby talus in Zigui County (30°53'N, 110°49'E), Hubei Province, China. These hosts belong to dwarf tree/shrub varieties (*Sapium sebiferum*, *Alangium chinense*, *Debregeasia orientalis*, *Boehmeria nivea*, *Alchornea davidii*) and vines (*Parthenocissus dalzielii*, *Vitis amurensis*, *Ampelopsis delavayana*). Zigui County is located in the Three Gorges, with steep slopes near the Yangtze River and its tributaries. This region is dominated by subtropical monsoon, with annual precipitation of 1490 mm and a yearly temperature of 17°C–19°C (Li et al., 2019). Under such a climate, *C. chinensis* begins to grow in mid-April and is about one month later than its hosts.

The aboveground part of *C. chinensis* (mainly stems) and leaves of hosts were used for lipid extraction. Detailed information on the lipid extraction, fractionation, and analysis procedures is identical to that of Zhao et al. (2018). Briefly, plant samples (ca. 2 g dry weight) were freeze-dried, ground, and ultrasonically extracted with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9:1, V/V). Then the extract was fractionated into aliphatic, aromatic, and polar fractions using silica gel column chromatography, and *n*-alkanes were eluted in the aliphatic fraction. *n*-Alkanes were quantified using a Shimadzu GC-2010 gas chromatograph (GC) equipped with a flame ionization detector. The δ<sup>13</sup>C values of individual *n*-alkanes were determined by using a Finnigan Trace GC attached to a Finnigan Delta Plus XP isotope ratio mass spectrometer, while the δ<sup>2</sup>H values of individual *n*-alkanes were analyzed by using a Trace GC-thermal conversion-isotope ratio mass spectrometer (Thermo Delta V advantage). All δ<sup>13</sup>C and δ<sup>2</sup>H values were reported in the notation (‰) relative to the VPDB standard and VSMOW standard, respectively.

### 3 Results

*n*-Alkanes in these plant samples mainly range from C<sub>23</sub> to C<sub>33</sub>, showing a strong odd over even predominance (Fig. 1, Table 1). Among the hosts, three (*D. orientalis*, *B. nivea*, and *V. amurensis*) have a C<sub>max</sub> (the homolog with the highest concentration) of C<sub>31</sub>, while C<sub>29</sub> dominates in the other hosts. Unlike the hosts, distributions of *n*-alkanes in *Cuscuta* are consistent, with the C<sub>29</sub> taken > 75% of the total concentration. Additionally, the concentrations of *n*-alkanes in *Cuscuta* are unexpectedly high, even higher than that in their hosts, with a maximum concentration of up to 1521.9 μg/g dry weight.

Since *n*-C<sub>29</sub> is abundant in both hosts and *Cuscuta*, this homolog is selected for the comparison of compound-specific carbon and hydrogen isotope compositions (Fig. 2). The δ<sup>13</sup>C values of C<sub>29</sub> (δ<sup>13</sup>C<sub>29</sub>) in *Cuscuta* range from –30.7‰ to –36.4‰, which are less negative than those in their hosts (–33.2‰ to –37.1‰). The difference of δ<sup>13</sup>C<sub>29</sub> in the paired host-*Cuscuta* has a mean value of 1.8‰. Besides, the δ<sup>2</sup>H values of C<sub>29</sub> (δ<sup>2</sup>H<sub>29</sub>) in *Cuscuta* vary between –225‰ and –244‰, which are negative than

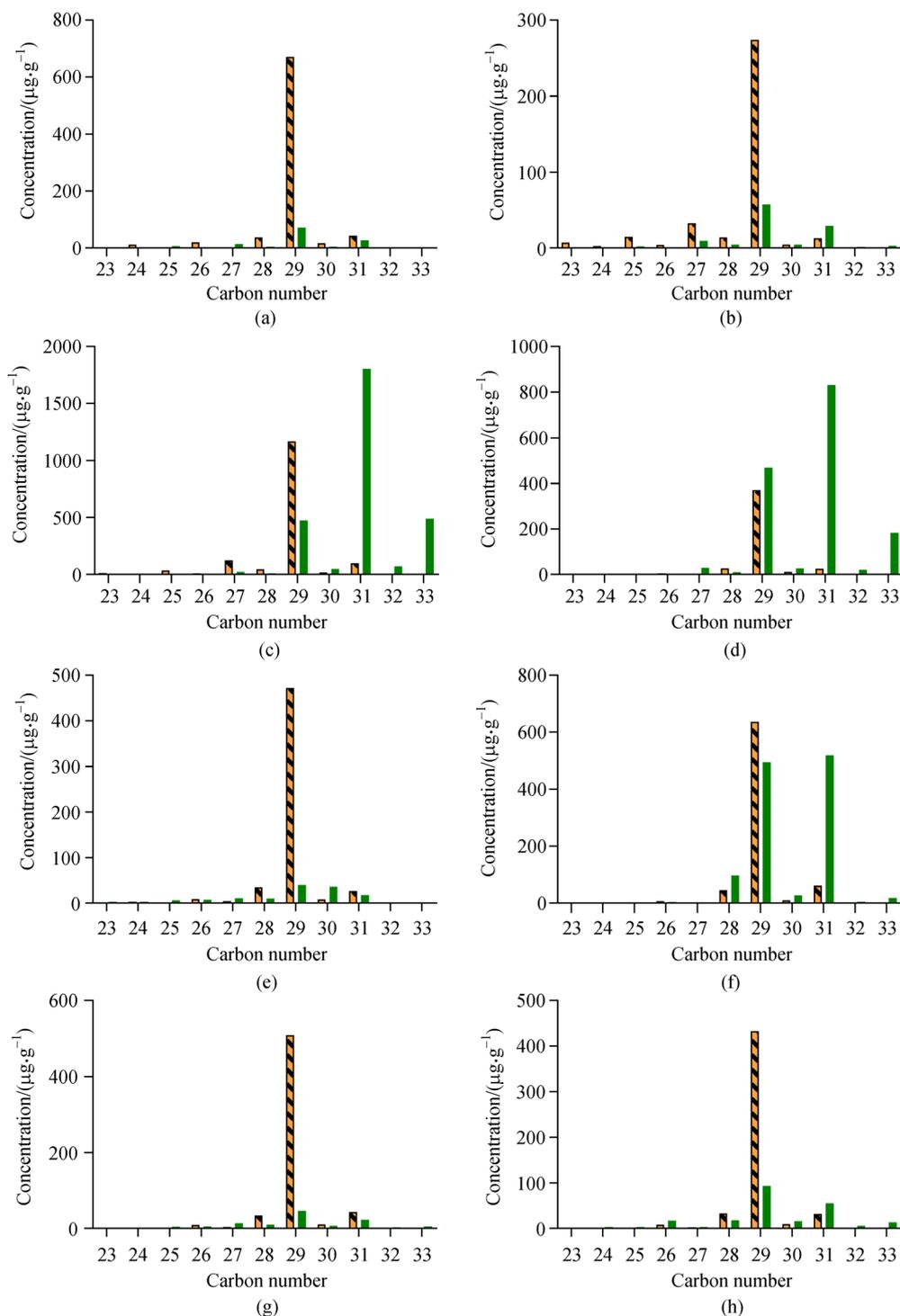
those in their hosts (–176‰ to –200‰). The difference of δ<sup>2</sup>H<sub>29</sub> in the paired host-*Cuscuta* averages –48‰. Furthermore, the δ<sup>13</sup>C<sub>29</sub> values in *Cuscuta* are strongly correlated with those in their hosts ( $R^2 = 0.73$ ,  $p < 0.01$ ), whereas the δ<sup>2</sup>H<sub>29</sub> values do not correlate between the host-*Cuscuta* pairs.

### 4 Discussion

As an important component of leaf epicuticle, leaf waxes are commonly attributed to maintaining water balance (Eglinton and Hamilton, 1967; Koch and Ensikat, 2008). For parasitic plants, the transpiration rates are relatively high (Ehleringer et al., 1986). Herein the accumulation of a higher concentration of *n*-alkanes in the aboveground of *Cuscuta* may prevent water loss and reduce evaporation. It is a little strange that the specimens of *Cuscuta* in this study are dominated by a single homolog (C<sub>29</sub>), which is uncommon in autotrophic plants (Bush and McInerney, 2013). To date, data of *n*-alkane distributions in parasitic plants are quite limited. Thus, it is not easy to explain why *Cuscuta* preferentially synthesizes *n*-C<sub>29</sub> alkane. More works are required to investigate additional parasitic specimens and to explore their alkane synthesizing genes.

The host is the only carbon source for *Cuscuta*, which absorbs nutrients (sucrose, water, etc.) that the host transports from leaves to stems. The δ<sup>13</sup>C values of parasite biomass are almost identical to those of the hosts (Ziegler, 1995). In this study, the δ<sup>13</sup>C<sub>29</sub> values in all *Cuscuta* specimens are less negative than those in their hosts. The differences of δ<sup>13</sup>C<sub>29</sub> values between *Cuscuta* and hosts in seven of the eight pairs > 1.5‰, far larger than the instrumental error (Fig. 2). Such a <sup>13</sup>C-enrichment of C<sub>29</sub> in *Cuscuta* probably results from the carbon isotope fractionation among plant organs. In plants, sucrose is the main form of nutrient transport that provides energy (O'Leary, 1981). Along with the transport, the δ<sup>13</sup>C values of sucrose increase from leaves to stems and to roots (Gleixner et al., 1993; Badeck et al., 2005). Therefore, the less negative δ<sup>13</sup>C<sub>29</sub> values in *Cuscuta* than those in its hosts probably result from the uptake of carbon nutrient from stems rather than from leaves. Such a close connection between carbon in the heterotrophic plant and its host is further supported by the strong correlation of δ<sup>13</sup>C<sub>29</sub> values between *Cuscuta* and its host.

Different from δ<sup>13</sup>C<sub>29</sub>, δ<sup>2</sup>H<sub>29</sub> values are lower in *Cuscuta* than in its hosts. During the synthesis of *n*-alkanes in plants, there are three major influences which determine the δ<sup>2</sup>H values of *n*-alkanes: 1) the δ<sup>2</sup>H values of plant's source water (Sachse et al., 2012); 2) influence of evapotranspiration on leaf water (Kahmen et al., 2013); 3) the δ<sup>2</sup>H values of the precursor molecule produced by the decomposition of sucrose and the associated central metabolic pathways (Newberry et al., 2015; Cormier et al., 2018). Plant life forms and species differences may result



**Fig. 1** Distribution of *n*-alkanes in *Cuscuta* and its hosts. The yellow twill-filled bars indicate *Cuscuta*, and the green bars indicate their hosts. (a) *Sapium sebiferum*; (b) *Alangium chinense*; (c) *Debregeasia orientalis*; (d) *Boehmeria nivea*; (e) *Alchornea davidii*; (f) *Parthenocissus dalzielii*; (g) *Vitis amurensis*; (h) *Ampelopsis delavayana*.

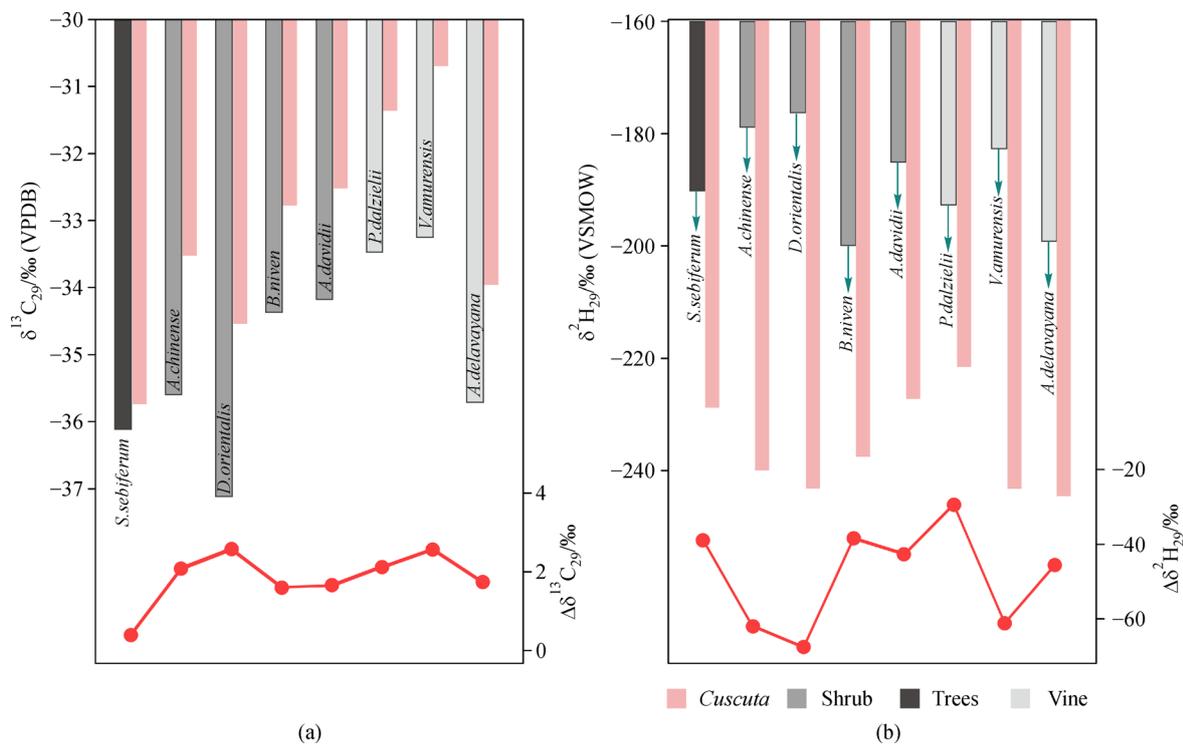
in different  $\delta^2\text{H}_{29}$  signatures in the host plants, which could be inherited into the heterotrophs (Sachse et al., 2012). For the more negative  $\delta^2\text{H}_{29}$  in *Cuscuta* than in its hosts, we speculate the following two main reasons. First, *Cuscuta* absorbs water from the host stem, which is less affected by

evapotranspiration than the host leaves. In contrast, the host leaves utilize  $^2\text{H}$ -enriched leaf water for *n*-alkane synthesis (Kahmen et al., 2013). Cormier et al. (2019) observed that  $\delta^2\text{H}$  values of leaf water in the host (*Hedera helix*) were about 15‰ higher than those of the parasite

**Table 1** Concentration ( $\mu\text{g/g}$ ) of *n*-alkanes in the host-*Cuscuta* pairs

Sample pair	Species	C <sub>23</sub>	C <sub>24</sub>	C <sub>25</sub>	C <sub>26</sub>	C <sub>27</sub>	C <sub>28</sub>	C <sub>29</sub>	C <sub>30</sub>	C <sub>31</sub>	C <sub>32</sub>	C <sub>33</sub>	Sum	ACL <sup>a</sup>	CPI <sup>b</sup>
Pair-1	<i>Cuscuta</i>	0.0	12.6	1.6	19.6	1.2	37.4	670.9 <sup>c</sup>	16.5	43.6	0.5	1.3	805.2	28.9	8.3
	<i>Sapium sebiferum</i>	1.5	1.5	6.1	2.9	14.2	5.0	<b>71.9</b>	4.7	27.0	1.2	3.3	139.4	28.9	8.0
Pair-2	<i>Cuscuta</i>	7.1	2.3	14.9	4.1	32.6	13.8	<b>273.9</b>	4.2	13.0	0.0	0.0	365.8	28.5	14.0
	<i>Alangium chinense</i>	0.6	0.5	2.1	1.1	9.5	4.2	<b>57.5</b>	4.7	29.2	1.4	2.9	113.8	29.3	8.4
Pair-3	<i>Cuscuta</i>	13.8	3.5	34.7	10.0	124.2	45.6	<b>1171.6</b>	18.6	99.9	0.0	0.0	1521.9	28.8	18.6
	<i>Debregeasia orientalis</i>	2.0	0.8	5.7	0.9	21.8	8.6	475.2	46.8	<b>1804.1</b>	70.9	491.5	2930.9	31.0	20.0
Pair-4	<i>Cuscuta</i>	0.0	1.0	0.3	5.5	2.2	26.5	<b>370.0</b>	10.7	25.9	0.4	0.4	442.9	29.0	9.0
	<i>Boehmeria nivea</i>	0.7	0.4	3.7	2.4	29.6	10.5	469.3	27.0	<b>831.5</b>	20.3	182.3	1577.6	30.5	23.6
Pair-5	<i>Cuscuta</i>	0.0	2.7	1.4	8.7	4.7	34.8	<b>472.4</b>	8.4	26.7	0.3	0.4	560.4	29.0	9.2
	<i>Parthenocissus dalzielii</i>	3.2	3.5	6.6	7.7	10.9	10.3	<b>40.0</b>	36.4	17.6	1.6	1.6	139.8	28.7	1.3
Pair-6	<i>Cuscuta</i>	0.0	1.5	0.5	7.4	2.4	46.5	<b>635.6</b>	9.4	61.5	0.3	0.5	765.6	29.1	10.8
	<i>Vitis amurensis</i>	0.0	0.3	0.4	3.4	3.0	96.8	495.5	27.8	<b>520.3</b>	5.8	18.0	1171.3	29.9	7.7
Pair-7	<i>Cuscuta</i>	0.0	2.3	1.3	8.9	4.0	33.9	<b>508.8</b>	10.2	43.5	0.5	0.8	615.0	29.0	10.0
	<i>Ampelopsis delavayana</i>	1.8	2.2	4.9	5.7	13.6	9.9	<b>47.0</b>	6.9	22.9	3.1	5.4	123.7	28.9	3.3
Pair-8	<i>Cuscuta</i>	0.0	1.2	0.8	8.1	2.5	33.3	<b>432.7</b>	9.5	31.7	0.3	0.5	521.3	29.0	8.9
	<i>Alchornea davidii</i>	1.1	3.0	3.4	17.4	2.8	17.9	<b>93.7</b>	15.5	55.5	5.8	13.7	233.8	29.4	2.7

Note: <sup>a</sup>ACL =  $(\sum_{\text{odd}} C_n \times n) / \sum_{\text{odd}} C_n$ ; <sup>b</sup>CPI =  $\frac{1}{2} \times \left[ \left( \frac{C_{23} + C_{25} + C_{27} + C_{29} + C_{31}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}} \right) + \left( \frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}} \right) \right]$ ; <sup>c</sup> homolog with the maximum concentration.



**Fig. 2**  $\delta^{13}\text{C}_{29}$  values (a) and  $\delta^2\text{H}_{29}$  values (b) in *Cuscuta* (pink bars) and its hosts (black bars: tree; dark bars: shrub; gray bars: vine), together with the differences of  $\delta^{13}\text{C}_{29}$  and  $\delta^2\text{H}_{29}$  values between *Cuscuta* and its hosts.

(*Orobranche hederæ*).

Alternatively, the  $^2\text{H}$ -depletion of  $\text{C}_{29}$  in *Cuscuta* may result from the different growth periods between *Cuscuta* and its host, which leads to the different  $\delta^2\text{H}$  value of the raw material for the synthesis of *n*-alkanes. In broad-leaved angiosperms, leaf waxes are normally synthesized in the early stage of leaf development (e.g., Kahmen et al., 2011; Tipple, 2013). In this case, the leaves of hosts tend to uptake stored  $^2\text{H}$ -enriched sugars for the synthesis of *n*-alkanes (Newberry et al., 2015). *Cuscuta* occupies when the leaves of hosts have been matured. Herein the heterotrophic plants can use the newly formed photosynthetic products, which is  $^2\text{H}$  depleted relative to the stored carbohydrates (Newberry et al., 2015; Cormier et al., 2018).

The pattern of  $^2\text{H}$ -depletion of *n*-alkanes in *Cuscuta* is quite different from the report of Cormier et al. (2019), which observes a consistent  $^2\text{H}$ -enrichment. Such a contrary may result from the species differences. The specimens in Cormier et al. (2019) belong to root parasites or mycoheterotrophic plants. The latter acquires solutes from hosts via fungi. In contrast, *Cuscuta* is a stem parasitic plant in this study. We could not exclude the influence of environmental conditions. This study was conducted in a humid subtropical climate, while Cormier et al. (2019) investigated specimens from tropical rainforest or temperate forest. Anyway, this study highlights that there are diverse  $^2\text{H}$  patterns in parasite-host pairs, and consolidates the (paleo)ecological potential of leaf wax  $\delta^2\text{H}$  values. Certainly more works are required to explore the distributions of *n*-alkanes from more parasitic plants and from diverse geographical settings, particularly under greenhouse conditions, to elucidate the relationship between *n*-alkanes and environmental factors. It is worthy of inferring whether the molecular and isotopic signatures can be transferred to sedimentary archives.

## 5 Conclusions

By comparing the molecular, carbon and hydrogen isotope compositions of leaf wax *n*-alkanes in parasitic plants and their hosts, the main findings are as follows:

1) the concentration of *n*-alkanes is quite high in *Cuscuta* and is preferentially dominated by  $\text{C}_{29}$  *n*-alkane. *Cuscuta* may synthesize more *n*-alkanes to keep the plant hydrated.

2) the  $\delta^{13}\text{C}_{29}$  values in *Cuscuta* are more positive than those in its hosts, possibly because of the carbon isotope fractionation of sucrose from leaves to stem in hosts.

3) the  $\delta^2\text{H}_{29}$  values in *Cuscuta* are more negative than those in its hosts, and the reasons may result from the utilization of stem water with less influence from evapotranspiration, and/or the use of newly synthesized carbohydrates which is  $^2\text{H}$ -depleted relative to stored sugars.

The above results infer the importance of plant nutrient status on the molecular and isotopic compositions of leaf waxes, which is worthy of exploring further whether this process affects the paleo applications of leaf wax ratios in ancient archives.

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## References

- Badeck F W, Tcherkez G, Nogués S, Piel C, Ghashghaie J (2005). Post-photosynthetic fractionation of stable carbon isotopes between plant organs—a widespread phenomenon. *Rapid Commun Mass Spectrom*, 19(11): 1381–1391
- Bush R T, McInerney F A (2013). Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. *Geochim Cosmochim Acta*, 117: 161–179
- Cormier M A, Werner R A, Sauer P E, Gröcke D R, Leuenberger M C, Wieloch T, Schleucher J, Kahmen A (2018).  $^2\text{H}$ -fractionations during the biosynthesis of carbohydrates and lipids imprint a metabolic signal on the  $\delta^2\text{H}$  values of plant organic compounds. *New Phytol*, 218(2): 479–491
- Cormier M A, Werner R A, Leuenberger M C, Kahmen A (2019).  $^2\text{H}$ -enrichment of cellulose and *n*-alkanes in heterotrophic plants. *Oecologia*, 189(2): 365–373
- Eglinton G, Hamilton R J (1967). Leaf epicuticular waxes. *Science*, 156 (3780): 1322–1335
- Eglinton T I, Eglinton G (2008). Molecular proxies for paleoclimatology. *Earth Planet Sci Lett*, 275(1–2): 1–16
- Ehleringer J R, Cook C S, Tieszen L L (1986). Comparative water use and nitrogen relationships in a mistletoe and its host. *Oecologia*, 68 (2): 279–284
- Gleixner G, Danier H J, Werner R A, Schmidt H L (1993). Correlations between the  $^{13}\text{C}$  content of primary and secondary plant products in different cell compartments and that in decomposing basidiomycetes. *Plant Physiol*, 102(4): 1287–1290
- Kahmen A, Dawson T E, Vieth A, Sachse D (2011). Leaf wax *n*-alkane  $\delta\text{D}$  values are determined early in the ontogeny of *Populus trichocarpa* leaves when grown under controlled environmental conditions. *Plant Cell Environ*, 34(10): 1639–1651
- Kahmen A, Hoffmann B, Schefuß E, Arndt S K, Cernusak L A, West J B, Sachse D (2013). Leaf water deuterium enrichment shapes leaf wax *n*-alkane  $\delta\text{D}$  values of angiosperm plants II: observational evidence and global implications. *Geochim Cosmochim Acta*, 111: 50–63
- Koch K, Ensikat H J (2008). The hydrophobic coatings of plant surfaces: epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. *Micron*, 39(7): 759–772
- Li C, Fu Z, Wang Y, Tang H, Yan J, Gong W, Yao W, Criss R E (2019). Susceptibility of reservoir-induced landslides and strategies for increasing the slope stability in the Three Gorges Reservoir Area: Zigui Basin as an example. *Eng Geol*, 261: 105279

- Liu J, An Z (2020). Comparison of different chain *n*-fatty acids in modern plants on the Loess Plateau of China. *Front Earth Sci.*
- Mishra J S (2009). Biology and management of *Cuscuta* species. *Indian J Weed Sci*, 41: 1–11
- Newberry S L, Kahmen A, Dennis P, Grant A (2015). *n*-Alkane biosynthetic hydrogen isotope fractionation is not constant throughout the growing season in the riparian tree *Salix viminalis*. *Geochim Cosmochim Acta*, 165: 75–85
- O'Leary M H (1981). Carbon isotope fractionation in plants. *Royal Society of New Zealand Wellington New Zealand*, 20(4): 553–567
- Pu Y, Jia J H, Cao J C (2018). The aliphatic hydrocarbon distributions of terrestrial plants around an alpine lake: a pilot study from Lake Ximencuo, Eastern Qinghai-Tibet Plateau. *Front Earth Sci*, 12(3): 600–610
- Sachse D, Billault I, Bowen G J, Chikaraishi Y, Dawson T E, Feakins S J, Freeman K H, Magill C R, McInerney F A, Van der Meer M T, Polissar P, Robins R J, Sachs J P, Schmidt H, Sessions A L, White J W C, West J B, Kahmen A (2012). Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annu Rev Earth Planet Sci*, 40 (1): 221–249
- Sessions A L (2016). Factors controlling the deuterium contents of sedimentary hydrocarbons. *Org Geochem*, 96: 43–64
- Tipple B J (2013). Capturing climate variability during our ancestors' earliest days. *Proc Natl Acad Sci USA*, 110(4): 1144–1145
- Zang J, Lei Y, Yang H (2018). Distribution of glycerol ethers in Turpan soils: implications for use of GDGT-based proxies in hot and dry regions. *Front Earth Sci*, 12(4): 862–876
- Zhao B, Zhang Y, Huang X, Qiu R, Zhang Z, Meyers P A (2018). Comparison of *n*-alkane molecular, carbon and hydrogen isotope compositions of different types of plants in the Dajiuhe peatland, central China. *Org Geochem*, 124: 1–11
- Ziegler H (1995). Deuterium content in organic material of hosts and their parasites. In: Schulze E D, Caldwell M, eds. *Ecophysiology of Photosynthesis*. Ecological Studies, Vol 100. Berlin: Springer, 393–408